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Fiber Type–Specific Adaptations to Exercise Training in Human Skeletal Muscle: Lessons From Proteome Analyses and Future Directions

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ABSTRACT

Skeletal muscle is a key determinant of sports performance. It is a highly specialized, yet complex and heterogeneous tissue, comprising multiple cell types. Muscle fibers are the main functional cell type responsible for converting energy into mechanical work. They exhibit a remarkable ability to adapt in response to stressors, such as exercise training. But while it is recognized that human skeletal muscle fibers have distinct contractile and metabolic features, classified as slow/oxidative (type 1) or fast/glycolytic (type 2a/x), less attention has been directed to the adaptability of the different fiber types. Methodological advancements in mass spectrometry-based proteomics allow researchers to quantify thousands of proteins with only a small amount of muscle tissue—even in a single muscle fiber. By exploiting this technology, studies are emerging highlighting that muscle fiber subpopulations adapt differently to exercise training. This review provides a contemporary perspective on the fiber type–specific adaptability to exercise training in humans. A key aim of our review is to facilitate further advancements within exercise physiology by harnessing mass spectrometry proteomics.

1 | Introduction

Skeletal muscle is an important determinant of health and physical capability. It exhibits a remarkable ability to adapt in a task-dependent manner, not only affecting its phenotypic characteristics but also having implications for whole body metabolism and function [1–3]. Muscle activity counters a wide range of metabolic disorders [4], age-related loss of muscle size [5], function [6], and predicts all-cause mortality [7]. Athletes exploit the adaptability of muscle when designing and planning their training to optimize performance within a specific task domain [8–11]. Therefore, understanding the mechanisms driving the phenotypic properties of skeletal muscle is of utmost importance in exercise physiology [12–15].

Skeletal muscle is a heterogeneous tissue composed of multinucleated muscle fibers interspersed with various cell types, including adipocytes, satellite cells, and endothelial cells. Muscle fibers, which constitute the majority of muscle tissue, are classified into slow (type 1) and fast fibers (type 2a and 2x) based on their expression of myosin heavy chain isoforms (MYH) [16]. These fiber types exhibit distinct contractile and metabolic properties. Type 1 fibers contract slowly, are fatigue-resistant, and rely on oxidative enzymes for energy production,

whereas type 2 fibers contract quickly, are more susceptible to fatigue, and primarily utilize glycolytic enzymes for energy production. Fiber-type composition has implications for muscle wasting conditions [17], metabolic disorders [18], aging [19], and sport performances [20]. For instance, the predominance of type 2 fibers in sprinters and type 1 fibers in endurance athletes highlights that fiber types contribute to driving a specific athletic phenotype [21, 22]. Furthermore, although fiber-type composition is largely genetically determined, denervation, bed rest, and exercise training can induce transitional shifts. Thus, muscle fiber type–specific analyses provide valuable information pertaining to pathophysiology [17, 19, 23] and in developing effective training programs, optimizing performance, and rehabilitation strategies for athletes [16, 24, 25].

Mass spectrometry (MS)-based proteomics, the large-scale study of proteins within biological systems, has become increasingly important for understanding complex molecular mechanisms and now enables researchers to interrogate thousands of proteins in exceedingly small amounts of material—even at the level of a single cell [26–28]. This allows researchers to uncover subtleties in muscle physiology and decipher the distinct fiber-type adaptations to various stimuli such as exercise [24, 29]. Harnessing MS-proteomics, studies have revealed unexpected fiber type–specific features of muscle aging, mitochondrial specialization, and exercise responsiveness [19, 24, 29, 30]. By leveraging the power of single-fiber proteomics, researchers can gain a deeper understanding of the fiber type–specific responses to exercise training, which is of relevance for developing targeted interventions to enhance muscle function and performance.

In this article, we highlight how recent advancements in MS-proteomics can uncover the fiber type–specific nature of adaptations to exercise training and discuss how this technology can be extended to decipher the molecular signature of athletes, providing insights that could inform personalized training and rehabilitation strategies.

2 | MS-Proteomics of Whole Skeletal Muscle and Single Muscle Fibers

Performing MS-proteomics on skeletal muscle tissue requires a meticulous workflow—from tissue handling and data processing to statistical analyses (i.e., bioinformatics). In a common approach called "bottom-up" proteomics, proteins are enzymatically digested into smaller peptides, which are then separated by liquid chromatography (LC) to reduce sample complexity before being analyzed by MS. The MS generates spectra for the peptides, which are subsequently compared to in silico spectra derived from protein databases using specialized software. This process enables the identification and quantification of proteins present in the sample [26, 31]. Despite its effectiveness, MS-based proteomics of muscle tissue is challenging due to a wide dynamic range, where abundant sarcomeric proteins (e.g., actin, myosin, titin) obscure detection of low-abundant proteins, limiting proteome coverage [26, 29, 30, 32]. To circumvent this, researchers employ several strategies, including specialized sample preparation protocols, optimized LC–MS methods, deep proteome background libraries, and advanced computational tools to reduce signal-to-noise and maximize coverage [26].

Protein coverage can be substantially improved with peptide-level fractionation techniques. For instance, Deshmukh et al. employed OFFGEL peptide fractionation, which separates peptides by their isoelectric point, along with a longer chromatographic gradient (150 min) to identify > 8000 proteins across 12 fractions in mice triceps muscle [32]. Another approach involves sequential digestion with multiple enzymes (e.g., trypsin and LysC), which generate distinct peptide fractions. Subsequent analysis of these fractions led to the detection of > 4000 proteins in rodent muscle samples [33, 34]. Additionally, tandem mass tag (TMT)-based proteomics coupled with peptide level fractionation offers another powerful approach to enhance proteome coverage [35]. TMT labeling involves chemically tagging peptides, pooling them, and fractioning the pooled samples for MS analysis. This multiplexed analysis not only improves proteome depth but also provides a robust approach for comparative studies across multiple samples [36, 37]. For example, Goodman et al. fractionated 10-plex TMT peptide pools into 12 fractions and detected > 4500 proteins in rodent skeletal muscle [38]. Although these fractionation techniques significantly enhance proteome depth, they come with trade-offs. They increase LC–MS measurement time, costs, and potentially lower reproducibility. Researchers must carefully weigh these considerations when designing experiments to study skeletal muscle proteomes. Moreover, the depth of proteome coverage continues to improve as advancements in MS instrumentation, acquisition strategies, and data processing technologies evolve. These advancements now allow for in-depth analyses of muscle single fibers.

Although more labor-intensive and costly, fiber type–specific proteomics analysis offers several advantages over whole muscle analyses. They provide a purer analysis with less contamination from other cell types and allow for the detection of fiber type–specific adaptations to exercise that are not apparent in whole-muscle analyses [24, 29]. Early studies on single muscle fiber proteomics focused on freshly isolated fibers from rodent and human muscles [19, 30]. However, optimized workflows, using freeze-dried samples [24, 28, 29], greatly enhance research opportunities by enabling analysis of samples collected in longitudinal studies and of biobank samples from previous studies. Although initial work focused on pooled single fiber analyses to ensure adequate protein yield and coverage with older technology [29], recent studies underscore the capability of the latest MS techniques to capture the deep proteome in just one individual fiber [28]. This project, based on a highly reproducible chromatography and sensitive TimsTOF MS, involved analysis of 1038 fibers and revealed an impressive coverage of almost 3000 proteins [28]. With this efficient workflow, using a short 21-min chromatographic gradient, we can now analyze 60 single fibers/day with high coverage and reproducibility, highlighting the feasibility of single-cell proteomic interrogation for muscle fibers [28]. Recent findings even utilized a shorter 15-min gradient for high-throughput single muscle fiber proteomics; however, at a lower coverage of around 629 proteins in 53 single fibers [39].

Analysis on pooled versus individual fibers each has advantages and drawbacks. Pooling fibers is more cumbersome due to the need for more dissections, but it offers a more homogeneous and generalizable view of the fiber type population and reduces the number of samples and batches to analyze. In contrast, analyzing individual fibers preserves their unique proteome features but

becomes challenging for large-scale studies with limited access to LC–MS instrumentation and resources. For instance, analyzing 40 single muscle fibers from 8 individuals before and after exercise training results in 640 samples, adding challenges because of longer measurement time and increased costs. Nonetheless, recently by analyzing > 1000 single muscle fibers, we uncovered some notable findings that otherwise would not have been shown with fiber pools or bulk muscle analyses [28]. The study showed significant heterogeneity within each fiber type, in which MYHs were not the sole drivers of skeletal muscle fiber heterogeneity; variations in metabolic, ribosomal, and cell junction proteins also played an important role [28]. Furthermore, although we were able to distinguish clusters of type 1 and 2 fibers based on contractile and metabolic proteome profiles, type 2x fibers did not exhibit a distinct protein signature compared to other type 2 fibers [28].

Regardless of pooling or not, the workflow starts by dissecting fibers out of muscle biopsy specimens using fine forceps under a stereomicroscope [29]. Freeze-drying biopsies before dissection is beneficial, as it eases the separation of fibers from the specimen and allows the dissection procedure to take place at room temperature. Each dissected single fiber piece, from freeze-dried muscle, is typically only around 1.5–2 mm in length but is nevertheless sufficient for analysis. Alternatively, single fibers can be dissected from cryopreserved muscle biopsies after immersing them in a membrane permeabilizing solution [28]. For pooled fiber analysis, each dissected fiber is lysed in an appropriate buffer. A portion of the lysate is then used for fiber typing, either through immunoblotting with antibodies against MYH isoforms or via gel electrophoresis, whereas the remaining lysate is reserved for MS-proteomics. Alternatively, individual muscle fibers are lysed and prepared for MS analysis directly, eliminating the need for prior fiber typing, as MS analysis can accurately determine fiber types.

A critical aspect of pooled fiber analyses is ensuring that pools are pure, which can be validated during the MS-analysis by assessing the expression of MYH-isoforms [24, 29]. Type 1 fiber pools should be characterized by a high abundance of MYH7, whereas type 2 fiber pools should be characterized by a high abundance of MYH2 (Figure 1A, left panel). In addition, fiber pools should separate clearly in a principal component analysis (PCA), which is a statistical technique used to simplify large datasets (termed dimension reduction). It transforms the large set of features, in this case proteins, into fewer features that contain most of the information in the dataset. The further the fiber pools separate along component 1 (the dimensionally reduced component explaining most of the dataset), the better the separation (Figure 1A, right panel). To this end, we typically pool 20–40 typified fibers per biopsy sample to capture a representative distribution of type 1 and 2 fibers [24, 29] but fewer fiber segments per pool may suffice. Fiber heterogeneity, subject population, and preparation workflow significantly influence the resolution of fiber pool separation. As illustrated in Figure 1, contrasting outcomes from two independent studies demonstrate this variability—one yielding clearly delineated fiber type pools (Figure 1A), the other showing MYH-isoform heterogeneity without distinct boundaries [40] (Figure 1B).

For analysis of individual fibers, the fiber does not necessarily need to be divided into two pieces as the fiber can be typified based on MYH expression patterns during the MS-analysis [28]. Using this technique, Moreno-Justicia et al. found only a very few fibers lacked MYH7, and none exhibited 100% MYH2 expression. Based on MYH expression patterns, most fibers could be classified as pure type 1 (≈35%) or type 2a (≈37%) and several hybrid 2a/2x fibers (≈18%) with the remainder being hybrid 1/2a. This typification technique demonstrated excellent agreement with traditional techniques using immunoblotting [28]. Thus, a purely MS-based workflow can effectively be utilized to typify fibers, identify hybrid fibers, and quantify the proportion of MYH in individual fibers. In addition to its ability to classify fibers into discrete fiber types, MS-based workflows have the ability to classify fibers along a continuum—or in multidimensional space.

Collectively, MS-proteomics on pooled fibers is cost-effective and provides a comprehensive view of muscle fiber populations,

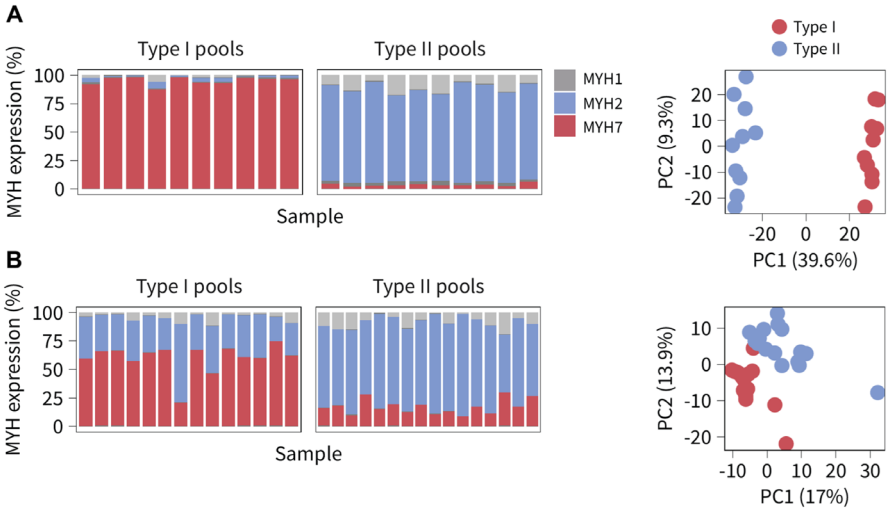

FIGURE 1 | Myosin heavy chain (MYH) isoform distribution in human muscle fiber type pools (vastus lateralis). (A) Fiber type pools with a homogenous MYH expression pattern that separate clearly in a principal component (PC) analysis along PC1. (B) Heterogenic fiber type pools with mixed MYH expression pattern that are not clearly separated in a PC analysis. Data based on Deshmukh et al. [29] (A) and Reisman et al. [40] (B).

making it ideal for large-scale studies. Although individual fiber analysis reveals unique inter-fiber proteome heterogeneity, it is resource-intensive.

3 | Bioinformatics

After proteins are quantified, a comprehensive bioinformatics workflow follows, encompassing quality control, batch effects correction, data management, statistical analyses, and data visualization. After initial quality control and batch correction, the data are filtered, and missing values are sometimes imputed based on their pattern. Each of these steps influences subsequent statistical analyses and outcomes.

Proteomics datasets often contain missing values, and a given protein might be quantifiable in only a subset of samples. This issue is particularly challenging in repeated measures designs or studies comparing multiple conditions. Consider a training study with pre- and post-muscle samples from the same individuals, representing a repeated measures design. In such a dataset, several proteins may only be quantifiable in either the pre- or post-sample. To mitigate loss of power and biologically relevant information from indiscriminate pairwise deletion, researchers typically employ a filtering strategy followed by imputation of remaining missing values [41]. The choice of filtering and imputation method offers distinct pros and cons and depends on the dataset, patterns of missing data, and research objectives [41]. In pre- to postintervention training studies, we and others have typically used a filtering criterion of 50%–70% valid values [24, 29]. Another approach is to filter for condition to reduce the risk of excluding features of biological relevance. For example, in tissues with large heterogeneity, such as between muscle fiber types, one can filter to accept 70% valid values in at least one condition. If applicable, missing data can be imputed by replacing missing values by random numbers drawn from the low end of a normal distribution [42] under the assumption that values are missing because of low abundance. Other imputation approaches can also be used, such as local methods using a weighted average of most similar peptides or proteins (i.e., K-nearest neighbor "KNN") and global methods as described in detail elsewhere [43].

Once the final dataset is prepared, it is analyzed to uncover meaningful biological patterns [44]. In many MS-proteomics studies, researchers conduct thousands of simultaneous statistical tests to identify differentially regulated proteins across conditions. Consequently, post hoc p value adjustments are necessary. For studies aiming to identify a few candidate targets, such as in biomarker discovery, stringent post hoc methods are appropriate. Conversely, for exploratory purposes, more sensitive methods can be chosen.

Statistical frameworks in omics studies typically regulate the false discovery rate (FDR), maintaining it below the conventional threshold of 0.05. Unlike raw p values, which estimate the probability of observing equal or more extreme effects under true null hypotheses for discrete features, FDR provides a more relevant metric by estimating the proportion of Type I errors within the pool of statistically significant findings. The Benjamini–Hochberg procedure is widely employed for this purpose [45]. It is a step-up method for p value adjustment, starting with the smallest p value and moving upward to determine which hypotheses can be rejected. However, the procedure can be overly conservative [46, 47] and increase the risk of false negatives (and Type II errors), especially when the number of comparisons is massive, the number of true null hypotheses is large, and when the sample size is small. Consider an experiment comparing the muscle proteome between two independent groups at $\alpha = 0.05$, covering 5000 proteins. In this scenario, the Benjamini–Hochberg procedure requires the top-ranked protein to have a p value smaller than 0.00001 to reject the null hypothesis. Even if we assume a 400% difference in protein abundance levels between groups and a massive effect size (Cohen's d) of 8.0, that is, change/difference relative to the pooled standard deviation, then approximately 200 participants would be needed in each group to detect a difference for the top-ranked protein. This is known as the "huge-scale" testing problem [48].

The Benjamini–Hochberg procedure also assumes tests are independent or positively dependent. Yet, proteins often exhibit complex dependencies, functioning as part of intricate networks. When data violate the assumption of independence or positive dependence, the Benjamini–Hochberg procedure inflates the risk of false discoveries [49]. Refined FDR adjustment procedures [46, 49, 50], including adaptive versions of the Benjamini–Hochberg procedure, permutation tests, and fusion methods that consider multiple data dimensions (e.g., effect size, variation, p value) [51], have since been developed that outperform the Benjamini–Hochberg procedure in terms of sensitivity [50, 52, 53]. Nonetheless, many studies continue to use this procedure, likely due to its simplicity, its status as one of the first FDR-correction methods, and unawareness of newer and refined approaches.

A well-established refined FDR method is the Storey and Tibshirani (2003) procedure, which adaptively controls the FDR [50]. This procedure estimates the proportion of true null hypotheses among all tested hypotheses based on the p value distribution and uses this to adapt FDR control, thereby enhancing the sensitivity in detecting true positives while still controlling the FDR. In human training interventions with sufficient effect response and a large distribution of small p values, the procedure is useful for FDR control [24, 54, 55]. For example, 4 weeks of endurance-based training was shown to significantly regulate 32 of 486 identified proteins (FDR ≤ 0.05) by MS-proteomics in human muscle lysates in already moderately trained individuals [54]—many of which otherwise would not have been inferred significant with the Benjamini–Hochberg procedure.

Permutation-based FDR [56] or fusion procedures [44, 51] can also be used depending on the distribution and pattern of the dataset. Permutation-based FDR is a method to control the false discovery rate by performing random permutations of the data to estimate the null distribution of test statistics. This approach does not rely on parametric assumptions about the data, making it particularly useful in situations where standard tests may not perform well (e.g., non-normal distributions or unequal variances). Fusion methods account for two dimensions of the data and typically integrate statistical significance (p value) with a magnitude of change or difference

($\log_2$-change, intensity, *Z*-score, etc.) [44, 51]. Such procedures are useful in studies with exceedingly large sample sizes, where statistical significance can be achieved without meaningful or clinically relevant changes, or in studies with small sample sizes but large effects [29, 51]. Fusion methods can also be combined with post hoc methods that adjust for multiple comparisons to identify interesting targets. For example, Blazev and Parker [57] used a combination of FDR-adjusted *p* value and fold change, based on a π-value [51], to uncover exercise-regulated phosphosites with the greatest magnitude of change and lowest adjusted *p* value in MS analyses of whole human muscle tissue.

Other than testing for differentially regulated proteins, analyses typically include enrichment and pathway analyses against established Gene Ontology (GO) terms or other databases, which categorize proteins based on their known functions and locations [58]. These analyses provide insights into the broader implications of which biological processes are activated or suppressed and identify changes in specific cellular components, and elucidate molecular functions such as enzymes and signaling pathways.

Two distinct methodological approaches are commonly employed for such analyses: over representation analysis (ORA) and gene set enrichment analysis (GSEA). ORA applies Fisher's exact test utilizing only the significantly regulated features, comparing their distribution to the whole detected feature space. In contrast, GSEA ranks all features according to their $\log_2$-fold change, followed by estimation of whether features of each GO term are significantly enriched at the top or bottom of the ranked list. GSEA offers particular value in instances where sample size or $\log_2$-fold changes are modest, as it can detect small (nonsignificant at the individual feature level) changes that are consistent across features within a GO term. This capability makes GSEA especially relevant for human studies and exercise interventions, where biological variability is often substantial and effect sizes may be subtle yet biologically meaningful.

Enrichment and pathway analyses can also be performed as a top–down approach before exploring statistical testing of all proteins of the dataset individually. In doing so, changes in structures, pathways, and functions are assessed broadly, followed by testing which proteins drive the change.

There is no single approach to bioinformatics of proteomic datasets and different approaches can lead to different outcomes. Advancements in proteomic techniques introduce challenges in selecting appropriate statistical tests to interpret the complex data generated. This is particularly true for single muscle fiber analyses comprising many hundreds, or even thousands, of fibers. Dimension reduction techniques, such as PCA and UMAP, can be employed to visualize and cluster the data effectively, whereas Pseudobulk approaches (i.e., methods) that aggregate single-cell data into "bulk-like" profiles, offer a robust framework for differential abundance analysis [28]. Despite these tools, there remains a pressing need to develop new analytical pipelines that can rigorously process and interpret large scale single muscle fiber datasets, ensuring accuracy and reliability of findings in this emerging area. As technologies advance, the integration of user-friendly, interoperable tools is essential for the effective analysis of complex datasets, facilitating new discoveries in muscle physiology.

4 | Muscle Fiber Type–Specific Response to Exercise Training

Although several studies over multiple decades have demonstrated notable fiber type–specific adaptations to different types of exercise training with immunoblotting techniques at a single-target level [59–64], only a few studies have yet exploited the technological breakthroughs in MS-based proteomics to study the muscle fiber type–specific responses to exercise training (Table 1).

Building on the seminal work from Murgia et al. [30], we applied a MS-proteomics workflow to explore the fiber type–specific response to exercise training in untrained but otherwise healthy men [29]. Over 12 weeks, the participants performed 1 h of moderate intensity cycling at 75%–90% heart rate max 4 times weekly (equivalent to zone 3–4 endurance training) [10]. We selected an untrained study population and a high-volume moderate intensity training to ensure an adequate training response and effect size. Before and after the training, we isolated 31–35 single fibers of the vastus lateralis, typified them by immunoblotting, and pooled them according to fiber type. The pools demonstrated clearly distinct MYH expression profiles that were homogeneous within each fiber type pool and separated clearly in a PCA (Figure 1A).

TABLE 1 | MS-proteomics of pooled human muscle fibers with exercise training studies.

Study	Intervention	Cohort	# Of pooled fibers	Coverage after filtering
Deshmukh et al. [29]	12-week, 1-h moderate-intensity cycling (zone 3–4), 4/week	5 M, untrained	31–35	3360
Reisman et al. [40]	8-week, 1–2-h low intensity cycling (zone 1), 3–4/week 8-week sprint training (4–8 × 30-s all out), 3–4/week	7–8 M, trained	$N = 6$, type 1 $N = 6$, type 2	1600
Moesgaard et al. [24]	12-week full-body resistance training 3/week	24 M/F, active	$N = 16 \pm 6$, type 1 $N = 21 \pm 6$, type 2	2883

Note: Training intensity zones based on Seiler [10].

Given the exploratory nature of the study with a small sample size ($n = 5$), but a large training effect size ($\log_2$-fold changes ranging −4.5 to 3.6), we applied a fusion method that combined raw p values with the $\log_2$ change of each protein as a post hoc method for p value adjustment [51] and performed thorough immunoblotting validation of significantly regulated proteins. Of 284 proteins exclusively identified in type 1 fibers, 61 of them were regulated by training, including well-described glycolytic and mitochondrial proteins, but also less described proteins, including MICU1, MICU2, and TANGO2. The latter is involved in transport and golgi organization and is implicated in a rare genetic disorder causing episodes of metabolic crisis with arrhythmias and muscle tissue breakdown, among other complications [65]. Of 124 proteins exclusively identified in type 2 fibers, 13 of them were regulated by training. The most significantly upregulated protein was DIABLO—a mitochondrial pro-apoptotic protein. Although this may seem counterintuitive, given the mitochondrial biogenic effect of endurance training, an upregulation of DIABLO likely reflects altered mitochondrial fission and fusion dynamics [66], which is a common feature of mitochondrial biogenesis and volume expansion incurred by training [67]. In this regard, TMEM70, which facilitates biogenesis and assembly of complex V [68], was among the most upregulated proteins in type 2 fibers. Thus, this early exploratory study revealed several novel exercise-regulated proteins exhibiting fiber-type specificity.

Using an optimized workflow involving highly reproducible chromatography, high-sensitivity MS, novel data acquisition strategies, and advanced computational pipelines, we have uncovered sex and fiber type–specific effects of resistance training [24]. This revealed pronounced enrichment of ribosomal proteins and myofilaments, specifically relating to small and large ribosomal subunits and initiation factors. Notably, resistance training upregulated RPS13 and RPL38, both of which are implicated in ribosomal specialization [24]. Although these proteomic changes could suggest a possible involvement of ribosomal subunit regulation in the response to resistance training, direct functional studies would be needed to confirm the precise role of these changes in translation regulation [24]. In addition, we found apparent enrichment of mitochondrial pathways pertaining to the transcription and translation of genes in type 2 fibers of males but not in type 1 fibers or in type 1 and 2 fibers of females [24].

Another interesting aspect of MS-proteomics is biomarker discovery across training modalities. A commonly shared exercise-responsive protein across training studies utilizing MS-proteomics is S100A13. It plays a role in the export of proteins and peptides, including FGF1 [69] and IL1-α [70], and in biological processes, such as angiogenesis and differentiation [71]. The consistent upregulation of S100A13 across various exercise training interventions and adrenergic stimuli [24, 54, 55] suggests this protein may be involved in muscle adaptation. Indeed, Lanfranchi et al. recently uncovered a potential link between S100A13 and Akt signaling following sprint interval training under hypoxic conditions [72].

Muscle responsiveness to training interventions can be influenced by multiple factors, including training intensity, duration, frequency, and individual variability. Although MS-based proteomics provides broad, high-throughput data, detecting subtle training adaptations can be challenging when effect sizes are small, variability is high, and sample size is limited. In a recent study, Reisman et al. [40] examined low-intensity training—approximating or slightly below intensity zone 1 [10]—in a group of 7–8 well-trained participants (VO_{2max}: 52 mL/min/kg), who exercised 3–4 times per week for 1–2 h over 8 weeks. To assess proteomic changes, the researchers employed TMT-based proteomics on pooled fiber segments from each fiber type, ultimately filtering down to a set of ~1600 proteins for downstream bioinformatics analysis.

The authors reported that the pooled fibers had heterogeneous MYH expression, hindering clear fiber-type discrimination [40]. Using the stringent Benjamini–Hochberg procedure for multiple testing correction, none of the proteins were differentially regulated by the low-intensity training. The lack of significant changes likely reflected the participants' high baseline fitness, fiber heterogeneity, and training regimen employed. This highlights the importance of matching the sample size and training intensity to the expected effect size and underscores the need for meticulous sample preparation strategies to ensure homogeneous fiber-type pools.

Despite only a few training studies being available, these have already uncovered several novel proteins that are regulated in a fiber-type dependent manner. The application of MS-proteomics to single-fiber analysis represents a significant advancement within exercise physiology. By enabling the detailed characterization of fiber type–specific adaptations, this approach provides new insights into the molecular mechanisms underlying muscle plasticity and performance.

5 | Perspectives and Future Directions

With the advancements in MS-proteomics, we are beginning to uncover the intricate molecular fingerprints of exercise in skeletal muscle [29, 54, 73, 74]. This powerful technology opens numerous avenues for exploration, allowing researchers to identify signature changes in response to various forms of exercise across diverse cohorts, from individuals with chronic diseases to elite athletes. Optimized and novel workflows lay a solid foundation for future studies to decipher the heterogeneity and spectrum of responsiveness across muscle fibers [28]. This will extend beyond measurements of protein abundance to include studying posttranslational modifications at the single fiber level and provide deeper insights into the molecular adaptation triggered by exercise. Moreover, complementary techniques will allow researchers to interrogate distinct cellular subtypes and sublocations within muscle tissue using microdissection methods [75], offering a more comprehensive understanding of exercise adaptation at the single-cell level (satellite cells, fibroblasts, etc.) and facilitate mapping the organelle-specific proteome to uncover the adaptive responses of subcellular structures such as mitochondria and sarcoplasmic reticulum. Finally, the emergence of deconvolution tools will allow researchers to infer muscle fiber-type composition directly from proteome data of mixed muscle tissue based on MYH intensity patterns.

Given the high-throughput nature of MS-proteomics at increasing coverage, well-designed studies with adequate sample size and sensitive workflows are key to making reliable inferences about the effects of exercise. This becomes critical in interventions involving athletes, where effect sizes are typically smaller compared to untrained individuals. With these technological advancements, we are excited to see how the field continues to evolve and uncover new dimensions of exercise biology at unparalleled resolution.

Author Contributions

M.H. and A.S.D. conceived and authored the paper. Both authors approved the final version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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